

Amendments to the Specification

Please amend the two consecutive paragraphs in the Brief Description of the Figures, beginning on line 8 of page 6 of the specification as follows:

Figures 1A, 1B, and 1C illustrate the flow cytometric analysis of cell populations in the Primary (1A) and Master (1B and 1C) ~~and Working~~ Cell Banks generated from a bone marrow aspirate following red blood cell lysis.

Figures 2A, 2B, and 2C illustrate the flow cytometric analysis of cell populations in the Primary (2A) and Master (2B and 2C) ~~and Working~~ Cell Banks generated from a bone marrow aspirate following density separation.

Please amend the paragraph beginning on line 12 of page 15 of the specification as follows:

In an additional embodiment, the invention includes a method of making a substantially homogenous cell population which co-express CD49c and CD90 comprising culturing a source of the cell population (e.g., human bone marrow cells) and selecting from the cultured source of the cell population, cells which co-express ~~CD40e~~ CD49c and CD90 by culturing the source of the cell population under low oxidative stress (e.g., glutathione, Vitamin C, Catalase, Vitamin E, N-Acetylcysteine). "Low oxidative stress," as used herein, refers to conditions of no or minimal free radical damage to the cultured cells.

Please amend the paragraph in Example 1, beginning on line 3 of page 26 as follows:

The generation of CFUs was monitored in 6-well plates concurrently initiated under identical conditions to the T75 flasks. The spent medium was removed from the 6-well plates and the adherent cells were fixed for 5 minutes in 100% methanol, and then stained with methylene blue to visualize the CFUs. An initial seeding density of 75,000 cells/cm² efficiently generated CFUs. After processing the bone marrow aspirate by either [[FICOLL[®]]] density gradient separation or ammonium chloride lysis, CFU efficiency was dramatically affected by oxygen concentration.

Please amend the paragraph in Example 1, beginning on line 18 of page 26 and bridging to page 27, line 8 of the specification as follows:

Resuspended cells (approximately 10^6) were aliquoted into 12x75 mm Flow Cytometry tubes and repelleted at 500 x g for 5 minutes. The HBSS was removed and 25 mL of the following antibodies (all obtained from Becton Dickinson), alone or in combination, were placed into each tube: mouse IgG1k FITC or -PE (clone MOPC 21) CD49c-PE (cl. C3II.1), CD90-FITC (cl. 5E10), CD45-FITC or -PE (cl. HI30). Tubes were gently vortexed and incubated for 30 minutes at 4°C. Cells were then washed in HBSS/1% bovine serum albumin, centrifuged (30 min, 4°C) and the resulting cellular pellet fixed by the addition of 250 microliters of 2% paraformaldehyde/HBSS. Flow cytometric analysis was performed employing a Becton Dickinson FACS Vantage SE cytometer and analyzed using CELLQUEST® software. Figure 1 depicts results representing data collected from 2,500-10,000 events per panel. After compensation for non specific antibody staining using mouse IgG1 isotype controls, cellular expression of CD45, CD49c and CD90 in the cultured bone marrow cells was assessed. The adherent population derived from mononuclear cells initially purified using ammonium chloride lysis contained approximately 70% CD49c positive cells at a similar stage of culture (Figure 1A). The majority of cells that did not express CD49c were positive for expression of hematopoietic/myeloid lineage marker CD45 (Figure 1A, LR quadrant), demonstrating that the CD49c positive cell population derived from human bone marrow isolated was not directly related to known hematopoietic precursors. ~~More than 94% of the adherent population was CD90 and CD49c positive (Figure 1B).~~

Please amend the paragraph in Example 2, beginning on line 11 of page 27 as follows:

Bone marrow cells were aspirated from the iliac crest of healthy adult human volunteers. The bone marrow aspirate was diluted with calcium and magnesium free phosphate buffered saline (PBS) to achieve a mononuclear cell concentration of 7×10^6 cells/mL and overlaid onto an equal volume of [[Histopaque® 1.119]] HISTOPAQUE® 1.119 (Sigma, St. Louis, Mo.) and centrifuged (30 min at 700 x g). The resulting mononuclear cell fraction was transferred to a clean centrifuge tube containing PBS and centrifuged (10 minutes at 500 x g). The cell pellet was re-suspended in PBS and centrifuged (10 minutes at 500 x g). The supernatant was aspirated from the cell pellet and the cells re-suspended in complete media.

Please amend the paragraph in Example 2, beginning on line 27 of page 27 and bridging to line 6 of page 28 of the specification as follows:

Cytometry analysis of the CFU generated showed that approximately 50% of the adherent population expressed the marker CD49c at 7 days in vitro (Figure 2A, sum of UL and UR quadrants). The majority of cells that did not express CD49c were positive for expression of hematopoietic/myeloid lineage marker CD45 (Figure 2A, LR quadrant), demonstrating that the CD49c positive cell population derived from human bone marrow isolated by this procedure was not directly related to known hematopoietic precursors. ~~More than 91% of the adherent population was CD90 and CD49c positive (Figure 2B).~~

Please amend the paragraph in Example 3, beginning on line 22 of page 28 of the specification as follows:

The purity of the cells (percentage of cells which co-express CD49c/CD90) in the Master Cell Bank was determined by flow cytometry using the same method as above. More than 94% of the adherent population was CD90 and CD49c positive (Figure 1B). The vast majority (>98%) of the resulting population expressed ~~CD90~~ CD49c (Figure 1C) and virtually lacked any expression of the myeloid related marker CD45 (Figure 1C, LR quadrant). Thus, the expansion procedure as described herein produces a substantially homogenous population of adherent cells which co-express CD49c and CD90 and lack significant expression of the marker CD45.

Please amend the paragraph in Example 3, beginning on line 1 of page 29 of the specification as follows:

Similarly, the master cell bank generated from the CFU derived using the method of Example 2 showed that more than 91% of the adherent population was CD90 and CD49c positive (Figure 2B) and the majority of cells (>98.8%) of the resulting cell population expressed ~~CD90~~ CD49c (Figure 2C) and virtually lacked any expression of the myeloid-related marker CD45 (Figure 2C, LR quadrant). Thus, the expansion procedure as described herein generates a substantially homogenous population of adherent cells which co-express CD49c and CD90 and lack significant expression of the marker CD45.